

# Protocol for measuring plasma membrane:cytoplasm fluorescence intensity ratios

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An abbreviated version of this protocol was published in Science Signaling in Nov 2019

Translocation of TRPV4-PI3K $\gamma$  complexes to the plasma membrane drives myofibroblast transdifferentiation

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## Detailed protocol

1. Open image in Image J.
2. Click Image → Color → Split Channels to separate fluorescence channels. Close all except for the channel you want.
3. Click the "straight line" icon in the toolbar.
4. Draw a line over the area to be measured on the cell, making sure to extend the line past the cell edge so it is easy to determine where the cell edge begins on the plot profile.
5. Click Analyze → Plot Profile.
6. Use the plot profile graph to determine roughly which pixels represent the cell edge, and which represent the cell interior.
7. Click "list" at the bottom left corner of the plot profile graph. This lists all of the gray intensity values on the graph for each pixel.
8. Click Edit → Select All → Copy, and paste into an excel spreadsheet.
9. Take an average of the gray intensity values of the cell edges (a few pixels), and the average of the gray intensity values of the cell interior (not including the cell edge).
10. Use these values to take a ratio of the cell edge intensity (plasma membrane) to the cell interior intensity (cytoplasm).

**How to cite:**(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Grove, L. and Olman, M. (2020). Protocol for measuring plasma membrane:cytoplasm fluorescence intensity ratios. Bio-protocol Preprint. [bio-protocol.org/prep256](https://bio-protocol.org/prep256).
2. Grove, L. M., Mohan, M. L., Abraham, S., Scheraga, R. G., Southern, B. D., Crish, J. F., Naga Prasad, S. V., Olman, M. A. and Prasad, S. V. N. (2019). Translocation of TRPV4-PI3K $\gamma$  complexes to the plasma membrane drives myofibroblast transdifferentiation . Science Signaling 12(607). DOI: [10.1126/scisignal.aau1533](https://doi.org/10.1126/scisignal.aau1533)

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